Supporting Online Material

Legends to Supplemental Figures

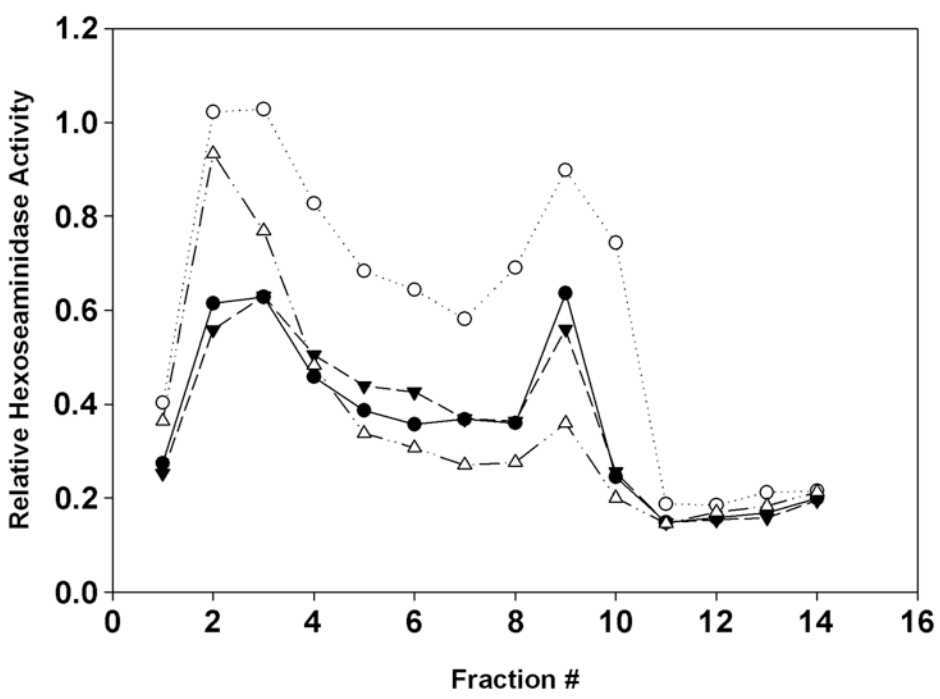
Supplemental Fig. 1. Induction of Ferroportin mediated-ferritin degradation does not result in ferritin localization in the lysosomes. HEK293T-Fpn cells were treated as in Figure 1A. Cells were harvested, homogenized and post nuclear supernatants (PNS) applied to a 30% Percoll gradient, which was centrifuged at 59,000 x g for 30 minutes. Gradients were fractionated into 1.0 ml fractions bottom to top (1-15) and each fraction was assayed for β-N-acetylhexoseaminidase (top panel) or ferritin (bottom panel). (●) Cells without FAC, (○) FAC loaded cells, (▼) FAC loaded + Ponasterone A, (Δ) FAC loaded+ Ponasterone A+ Chloroquine.

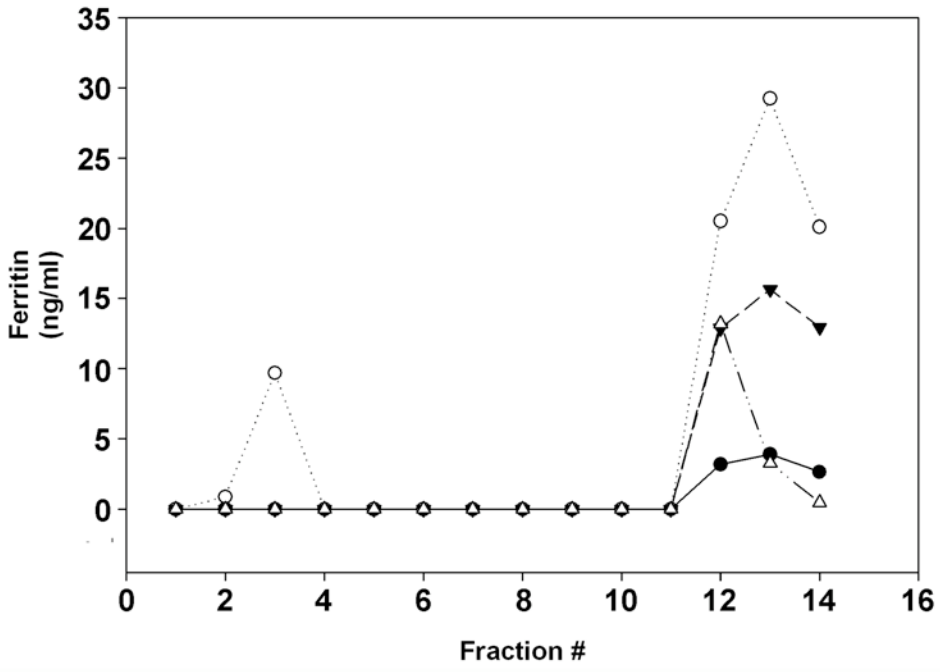
Supplemental Fig. 2. Ferritin in ts85 cells at the restrictive temperature is not ubiquitylated. FM3A and ts85 cells were treated as in Fig. 3A, ferritin was immunoprecipitated and examined by Western blot analysis using an antibody to ubiquitin.

Supplemental Fig. 3. Expression of human ferritin in *S. cerevisiae*. (**A**) Transformed cells were grown in medium with glucose (black bars) or galactose (grey bars) for 24 hours, cells were washed and cellular iron content determined using Atomic Absorption Spectroscopy. (**B**) Wt, Δ*ccc1* or Δ*ccc1*/pGAL-H+L containing yeast strains were serial diluted onto synthetic medium plates supplemented with glucose or galactose and 3 mM $FeSO_4$. Cells were incubated at 30ºC and growth assessed after 48 hours. (**C**) Wt strain cells, containing an integrated iron sensitive reporter construct (FET3*lacZ)* were transformed with either control vector (pGAL) or a ferritin containing vector (pGAL-H-ferritin). Cells were grown in medium with galactose for 24 hours, harvested, and β-galactosidase activity and protein levels determined. (**D**) Wt and Δ*ccc1* strains were transformed with pGAL or pGAL-H+L-ferritin. Transformed cells were grown in medium with galactose for 20 hours and then washed and incubated in either galactose or glucose medium for 15 hours. Cells were harvested, extracts heated to 75°C for 15 minutes and then applied to non-denaturing gel electrophoresis. The gels were stained

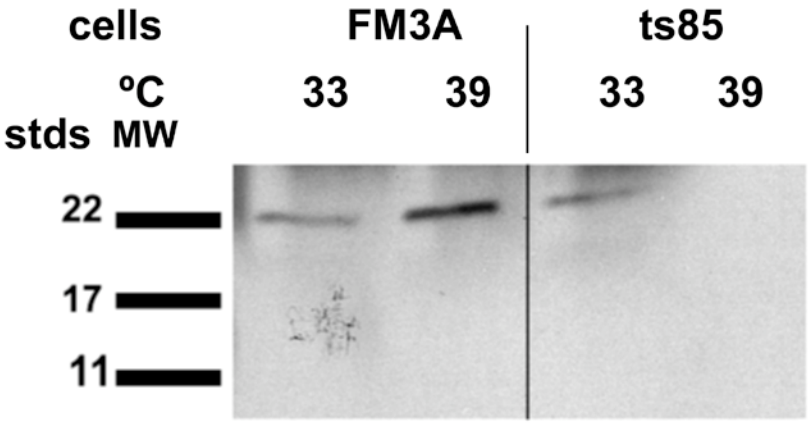
with Prussian blue or examined by Western blot analysis using an antibody to ferritin. Phosphoglucokinase (PGK) was used as a loading control.

Supplemental Figure 1





Supplemental Figure 2



Supplemental Figure 3